CASE REPORT

Is basaloid squamous carcinoma of the esophagus high-grade malignancy? A report of six cases

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Abstract

Basaloid squamous carcinoma of the esophagus (BSCE) is a rare malignant disease. We herein described six cases of BSCE treated by esophagectomy in our department, the clinicopathological features of which were analyzed. Only one of the six cases was diagnosed with BSCE prior to surgery. The depth of tumor invasion was T3 and T1b in 4 and 2 cases, respectively. Metastatic lymph nodes were detected in two cases of advanced carcinoma. All four cases of advanced carcinoma recurred and died of metastasis, and the period of disease-free survival and overall survival after surgery was 5-18 months and 24-42 months, respectively. On the other hand, as of 21 months and 52 months after surgery, two patients with superficial carcinoma have been free from recurrence. Immunohistochemistry revealed that the anti-phosphohistone H3 (PHH3) mitotic index (MI) was significantly higher and the p21 labeling index (LI) was significantly lower than those in conventional esophageal squamous cell carcinoma. Although BSCE immunohistochemically suggests high-grade malignancy, radical excision may contribute to a better outcome in the early stage.

Key words

Basaloid squamous carcinoma, Phosphohistone H3, p21

1 Introduction

Basaloid squamous carcinoma (BSC) is a distinct variant of squamous cell carcinoma (SCC), which first described as a laryngopharyngeal tumor by Wain *et al.* in 1986 [1]. Basaloid squamous carcinoma of the esophagus (BSCE) is a rare tumor, accounting for 0.4%-3.6% of all esophageal carcinomas [2]. BSCE is generally considered to have a poorer outcome than that of SCC because it is characterized by a poor degree of differentiation, high proliferative activity [3], high biological malignancy [4-6], and high incidence of distant metastasis.

Immunohistochemistry using various molecular biological markers, such as cytokeratin subtypes, p53, B-cell lymphoma 2 (bcl-2), c-myc, cyclin D1 (CCND1), and E-cadherin, have recently been examined in patients with BSCE [2, 6-10]; however, no consensus has been reached.

Histone H3 is one of the five histone proteins that together form the major protein constituents of chromatin in eukaryotic cells. Histone H3 (Ser10) is phosphorylated in association with mitotic chromatin condensation in the late G2 and M phases of the cell cycle [11]. Therefore, anti-phosphohistone H3 (PHH3) can be used as a specific mitotic marker. We previously demonstrated that the expression of PHH3 had an impact on the prognosis of patients with esophageal SCC [12].

p21 is a cyclin-dependent kinase inhibitor that directly inhibits the activity of the CCND1/CDK4 complex. We previously reported a correlation between a high p21 LI and good prognosis in SCC patients [13].

To the best of our knowledge, the value of PHH3 and p21 expression in BSCE has not been previously evaluated. We herein described six cases of BSCE treated by esophagectomy and analyzed their clinicopathological features and the expression of p21 and PHH3.

2 Case presentation

2.1 Materials and methods

2.1.1 Patients

In the present study, BSC was defined as the presence of atypical basaloid cells accounting for more than 50% of the tumor. Six (among 481 cases of esophageal SCC and adenocarcinoma, 1.2%) surgical cases were diagnosed pathologically as BSCE between April 1999 and March 2012 at Kyoto Prefectural University of Medicine (Kyoto, Japan).

2.1.2 Immunohistochemistry

Paraffin sections (3-μm thick) of tumor tissue were subjected to immunohistochemical staining for PHH3 and p21 using the avidin-biotin-peroxidase method. Briefly, paraffin sections were dewaxed in xylene and hydrated through a graded series of alcohols. Antigen retrieval was performed by heating the samples in Dako REAL Target Retrieval Solution (Glostrup, Denmark) for 40 min at 95°C. Endogenous peroxidase activity was quenched by incubating the sections for 30 min in 0.3% H_2O_2. The sections were treated with a protein blocker, and were then incubated for 1 hour at room temperature with the following antibodies: a PHH3 antibody (rabbit polyclonal PHH3 antibody [Ser 10], diluted 1:5,000, from Millipore, Billerica, MA, USA) and p21 antibody (rabbit polyclonal p21 antibody, diluted 1:1,000, from Cell Signaling Technology, Beverly, MA, USA). The avidin-biotin-peroxidase complex system (Vectastain ABC Elite kit, from Vector Laboratories, Burlingame, CA, USA) was used for color development with diaminobenzidine tetrahydrochloride. The sections were counterstained with hematoxylin and were subsequently dehydrated through a graded series of alcohols, cleared in xylene, and mounted. Control sections of known positive SCC were included in each antibody run, and negative control sections were produced by omitting the primary antibody.

The determination of proliferative activity by immunohistochemistry was performed quantitatively by counting immune-reactive tumor cells in the most intensely stained areas. The PHH3 mitotic index (MI) was calculated as the number of positive cells in 10 consecutive high-power fields (400×) in areas with the highest mitotic activity. Only distinct immunoreactive tumor cell nuclei were counted. p21-stained cells were quantified in 5 selected fields of the highest proliferative activity at 400× magnification. The LI of each case was calculated as the number of positive cells divided by the total number of examined cells in all examined fields.

2.1.3 Statistical analysis

Statistical analyses were carried out using the Student's *t*-test for comparisons between two groups. Differences were considered significant when the associated *p*-value was less than 0.05. All analyses were performed using statistical software (JMP, version 10; SAS Institute Inc., Cary, NC, USA).

2.2 Results

2.2.1 Clinicopathological features and postoperative courses

Table. Clinicopathological features and postoperative courses of six patients with BSCE

Case	1	2	3	4	5	6
Age/sex	71/F	73/M	68/M	51/M	67/M	67/M
Location	MtLt	AeLt	Lt	Mt	Mt	Mt
Macroscopic classification	Type3	Type3	Type3	Type2	II a+ II c	II a+ II c
Biopsy specimen	scc	scc+bsc	scc	scc	scc	scc
pT	T3	T3	T3	T3	T1b	T1b
pN	N0	N2	N1	N0	N0	N0
ly	-	+	+	-	-	-
v	+	+	+	+	-	+
pStage	II	III	III	II	I	I
Preoperative chemotherapy	-	-	UFT+CDDP	-	-	-
Postoperative adjuvant chemotherapy	-	CPT-11+CDGP	-	-	-	-
Chemotherapy after recurrence	docetaxel, paclitaxel, CPT-11	UFT	cisplatin plus 5-FU, docetaxel	cisplatin plus 5-FU	-	-
PHH3 MI	25	33	39	55	86	28
p21 LI	2.2	28.5	1.8	1.8	7.6	3.6
DFS (months)	5	18	9	7	52	21
OS (months)	29	24	42	25	52	21
Outcome	dead	dead	dead	dead	alive	alive

Note. The terms and definitions used follow the criteria of the Japanese Classification of Esophageal Cancer, 10th edition; The macroscopic classification used was according to endoscopic classification based on the guidelines for Clinical and Pathological Studies of the Japanese Society for Esophageal Disease; Mt: middle thoracic esophagus; Lt: lower thoracic esophagus; Ae: abdominal esophagus; DFS: disease-free survival; OS: overall survival; PHH3 MI: phosphohistone h3 mitotic index; LI: labeling index

The table shows the clinicopathological features and postoperative courses of the six patients. Their mean age was 66 years old. Five of the six patients were male. Regarding the location of the tumor, middle thoracic, lower thoracic and abdominal esophagus was 4, 1 and 1 cases, respectively. Only one of the six cases was diagnosed with BSC before surgery. Only 1 case of advanced carcinoma received preoperative chemotherapy. All six patients underwent curative subtotal esophagectomy and two-field lymphadenectomy. Microscopically, the major component was basaloid cells. Solid nests with comedo-type central necrosis and the deposition of a basement membrane-like material were identified (see Figure 1). A trabecular structure and cribriform pattern were also found. Regarding the depth of tumor invasion, T3 and T1b was 4 and 2 cases, respectively. Metastatic lymph nodes were detected in 2 cases of advanced carcinoma. The positive rate of vessel invasion was 100% (4/4) in advanced carcinoma and 50% (1/2) in superficial carcinoma. The positive rate of lymphatic invasion was 50% (2/4) in advanced carcinoma and 0% (0/2) in superficial carcinoma. One case of metastatic lymph nodes received postoperative adjuvant chemotherapy (CPT-11 plus CDGP). All four advanced carcinoma recurred and died of metastasis. Chemotherapy (cisplatin plus 5-FU [FP], docetaxel, paclitaxel, and CPT-11) after recurrence was not effective in any case. The median period from surgery to recurrence was 8 months (5-18 months). Initial metastatic lesions were detected in the lung (2 case), liver (1 case), and para-aortic lymph nodes (1 case). Overall survival after surgery in patients with advanced carcinoma was 24-42 months. On the other hand, as of 21 months and 52 months after surgery, two patients with superficial carcinoma have been free from recurrence.

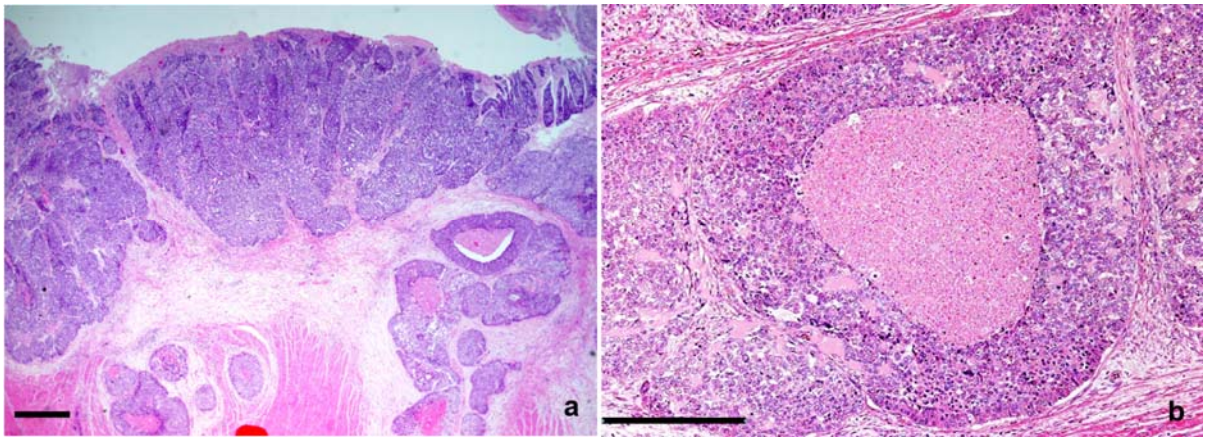

Figure 1. Microscopic examination of the excised tumor of case 1. (a) The tumor was composed of basaloid cells arranged in solid nests (HE staining; 20×, Bar = 500 μm). (b) Central necrosis (comedo-type necrosis) and deposition of a basement membrane-like material were observed (HE staining; 100×, Bar = 500 μm).

2.2.2 Immunohistochemistry

Figure 2 and 3 show the immunohistochemistry results of PHH3 and p21.

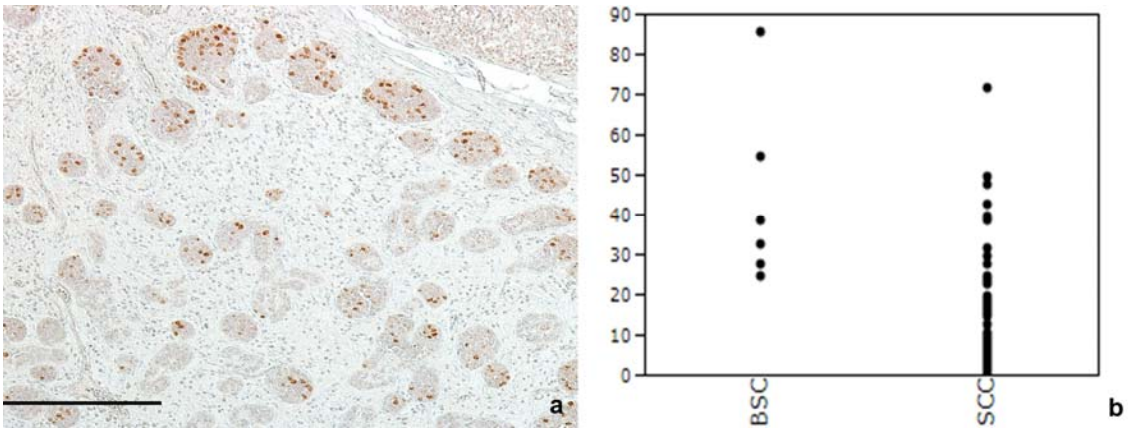

Figure 2. PHH3 mitotic index. (a) Immunohistochemical staining of a BSCE tumor sample with PHH3 antibodies (100×, Bar = 500 μm). (b) PHH3 MIs between BSCE samples and SCC samples [12]. Statistical analysis: Student's *t*-test. $p < .001$.

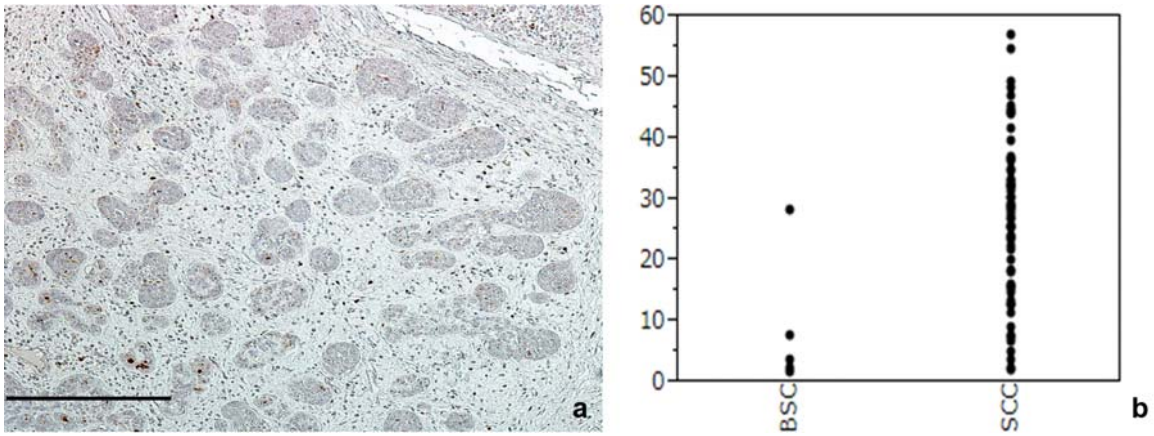

Figure 3. p21 labeling index. (a) Immunohistochemical staining of a BSCE tumor sample with p21 antibodies (100×, Bar = 500 μm). (b) p21 LIs between BSCE samples and SCC samples [13]. Statistical analysis: Student's *t*-test.

3 Discussion

BSC is an uncommon malignant epithelial neoplasm, which consists of small cells that resemble basal cells, that forms nests of various sizes or trabecular arrangement, and occasionally forms irregular adenoid or small cystic structures. BSC is also characterized by the deposition of basement membrane-like substances both within and outside tumor nests [2, 14]. BSCE has predominantly been observed in the middle thoracic esophagus in men [2, 3, 5]. In our series, five of six patients were male and tumors were mostly located in the middle part of the thoracic esophagus.

The growth pattern of BSCE is downward and expansive even at an early stage [15]. Superficial BSCE shows a submucosal tumor-like form or polypoid elevation [16, 17]. Even in advanced BSCE, tumors are frequently covered by a normal epithelium [9, 16]. Therefore, only 10% of BSCE cases are correctly diagnosed because of the difficulties associated with diagnosing BSCE by preoperative biopsy [16]. In our series, only one of six patients was diagnosed with BSCE before surgery. BSCE presents with high venous invasion because it primarily progresses in the stratum submucosa. Ishii *et al*. reported that the rates of venous invasion in patients who had superficial BSCE and BSCE with T2 or deeper invasion were 38% and 85%. The rate of venous invasion of BSCE was higher than the mean rate of superficial SCC (22.7%); however, no significant differences were observed for lymphatic invasion [16]. BSCE frequently progresses via hematogenous metastases rather than lymph node metastases [8, 17, 18]. In our series, the positive rate of vessel invasion was 100% (4/4) in advanced carcinoma and 50% (1/2) in superficial carcinoma. Hematogenous metastasis to organs such as the lung and liver occurred in three cases of advanced carcinoma.

In patients with SCC, we reported that a low PHH3 MI (cut-off value of 10) was correlated with good prognosis [12], while a high p21 LI (cut-off value of 30%) was also correlated with good prognosis [13]. In this study, the PHH3 MIs of BSCE were significantly higher than those of conventional SCC, and those of all BSCE cases were more than 10. Furthermore, the p21 LIs of BSCE was significantly lower than those of conventional SCC, and those of all BSCE cases were less than 30%. In this study, we demonstrated that BSCE exhibited high proliferative activity and high-grade malignancy.

No standard treatment has been established for BSCE. Combination chemotherapy with 5FU plus CDDP (FP) was previously shown to be effective in a neoadjuvant setting and recurrent BSCE [6, 10, 18]. Shibata *et al*. reported that taxan temporarily suppressed tumor progression of BSCE [10], but there was no report that it was effective for BSCE. In our series, postoperative adjuvant chemotherapy was performed in one of four advanced carcinoma cases. The patient who received postoperative adjuvant chemotherapy has been free from tumor recurrence for 18 months following surgery; however, tumor recurrence occurred within 5-9 months after surgery in the three cases that did not receive postoperative adjuvant chemotherapy. Therefore, postoperative chemotherapy may inhibit the recurrence of malignancy. Meanwhile, chemotherapy such as FP, taxan, and CPT-11 was not effective after tumor recurrence in our cases. The further accumulation of cases is necessary to establish effective and concrete protocols for such treatments.

BSCE generally has a poor outcome; however, a recent study has suggested that outcomes after curative resection may not differ significantly from those of common SCC [3, 8, 16, 19]. In a comparison of 60 cases of BSCE with typical SCC, Yoshioka *et al*. reported that the survival rates with early stage BSCE were similar to those of patients with common SCC. However, no long-term survivors were found among patients with advanced stage BSCE [20]. In our study, all four cases of advanced carcinoma recurred and died of metastasis. On the other hand, two patients with superficial carcinoma have been free from recurrence as of 21 months and 52 months after surgery. BSCE immunohistochemically suggests high-grade malignancy and has a poor outcome in the advanced stage of the disease; radical excision may contribute to a better outcome in the early stage.

Competing interests

The authors declare that they have no competing interests.

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